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=> s entner-doudoroff pathway

L1 680 ENTNER-DOUDOROFF PATHWAY

=> s L-amino acid and production

L2 1213 L-AMINO ACID AND PRODUCTION

=> s 12 and 11

L3 3 L2 AND L1

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L3 ANSWER 1 OF 3 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

TI Production of L-amino acid

comprises culturing microorganism, which is Gram-negative bacterium having Entner-Doudoroff pathway and which has been modified so that specific activities are enhanced.

AN 2004-212658 [20] WPIDS

AB US2003219882 A UPAB: 20040324

NOVELTY - **Production** of **L-amino acid** comprises culturing a microorganism having an ability to produce an

L-amino acid in a medium. The microorganism is a Gram-negative bacterium having the Entner-Doudoroff pathway and which has been modified so that 6-phosphogluconate dehydratase activity or 2-keto-3-deoxy-6-phosphogluconate aldolase

activity, or activities of both are enhanced.

DETAILED DESCRIPTION - Production of L-

amino acid comprises culturing a microorganism having an

ability to produce an L-amino acid in a

medium to produce and accumulate the L-amino

acid in the medium and collecting the L-amino

acid from the medium. The microorganism is a Gram-negative

bacterium having the Entner-Doudoroff pathway

and which has been modified so that 6-phosphogluconate dehydratase activity or 2-keto-3-deoxy-6-phosphogluconate aldolase activity, or

activities of both are enhanced. The L-amino acid is L-amino acid produced by a

biosynthetic pathway utilizing pyruvic acid as an intermediate.

USE - For producing an L-amino acid,

e.g. L-glutamic acid (preferably), L-arginine, L-glutamine, L-proline, L-leucine, L-isoleucine, L-valine, or L-alanine (claimed).

ADVANTAGE - The method improves productivity of L-amino acids in bacteria from a viewpoint different from known techniques.

Dwg.0/2

ACCESSION NUMBER:

2004-212658 [20] WPIDS

DOC. NO. CPI:

C2004-084256

TITLE:

Production of L-amino

acid comprises culturing microorganism, which is

Gram-negative bacterium having Entner-Doudoroff pathway and which has been

modified so that specific activities are enhanced.

DERWENT CLASS:

B05 D16 E19

INVENTOR(S):

ASANO, T; HARA, Y; IZUI, H; NAKAMATSU, T; WATANABE, Y

(AJIN) AJINOMOTO CO INC PATENT ASSIGNEE(S):

COUNTRY COUNT:

PATENT INFORMATION:

PATENT NO KIND DATE WEEK LA PG US 2003219882 A1 20031127 (200420)\* 12

#### APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 2003219882	A1	US 2003-396488	20030326

PRIORITY APPLN. INFO: US 2003-396488 20030326

ANSWER 2 OF 3 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN L3

ΤI Production of L-amino acid

> comprises culturing microorganism, which is Gram-negative bacterium having Entner-Doudoroff pathway and which

has been modified so that specific activities are enhanced;

gene overexpression enhancement for strain improvement and amino acid preparation

AN 2004-12299 BIOTECHDS

AΒ DERWENT ABSTRACT:

NOVELTY - Production of L-amino

acid comprises culturing a microorganism having an ability to produce an L-amino acid in a medium. The

microorganism is a Gram-negative bacterium having the Entner-

Doudoroff pathway and which has been modified so that

6-phosphogluconate dehydratase activity or 2-keto-3-deoxy-6-

phosphogluconate aldolase activity, or activities of both are enhanced.

DETAILED DESCRIPTION - Production of L-

amino acid comprises culturing a microorganism having

an ability to produce an L-amino acid in a medium to produce and accumulate the L-amino acid in the medium and collecting the L-amino acid from the medium. The microorganism is a Gram-negative bacterium having the Entner-Doudoroff pathway and which has been modified so that 6-phosphogluconate dehydratase activity or 2-keto-3-deoxy-6-phosphogluconate aldolase activity, or activities of both are enhanced. The L-amino acid is L-amino acid produced by a

biosynthetic pathway utilizing pyruvic acid as an intermediate.

USE - For producing an L-amino acid,

e.g. L-glutamic acid (preferably), L-arginine, L-glutamine, L-proline, L-leucine, L-isoleucine, L-valine, or L-alanine (claimed).

ADVANTAGE - The method improves productivity of L-amino acids in bacteria from a viewpoint different from known techniques. (12 pages)

ACCESSION NUMBER: 2004-12299 BIOTECHDS

TITLE: Production of L-amino

acid comprises culturing microorganism, which is

Gram-negative bacterium having Entner-Doudoroff pathway and which has been

modified so that specific activities are enhanced;

gene overexpression enhancement for strain improvement and

amino acid preparation

AUTHOR: HARA Y; IZUI H; ASANO T; WATANABE Y; NAKAMATSU T

PATENT ASSIGNEE: AJINOMOTO CO INC

PATENT INFO: US 2003219882 27 Nov 2003 APPLICATION INFO: US 2003-396488 26 Mar 2003

PRIORITY INFO: US 2003-396488 26 Mar 2003; US 2003-396488 26 Mar 2003

DOCUMENT TYPE: Patent

LANGUAGE: Facelic English

OTHER SOURCE: WPI: 2004-212658 [20]

ANSWER 3 OF 3 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN **Production** of L-amino acids in Gram-negative bacteria, useful particularly for making glutamic acid, by increasing activity of **Entner-Doudoroff pathway** enzymes;

involving Enterobacter agglomerans culture medium

AN 2004-00510 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - **Production** of L-amino acids (I) by culturing a Gram-negative bacterium that: (a) contains the **Entner- Doudoroff pathway**; and (b) has been modified so that activity of 6-phosphogluconate dehydratase (EDD) and/or 2-keto-3-deoxy-6-phosphogluconate aldolase (EDA) are increased, is new. (I) Is produced by a pathway that uses pyruvate as intermediate.

BIOTECHNOLOGY - Preferred Bacteria: These are enterobacteria, particularly an Enterobacter and specifically E. agglomerans ATCC 12287 or AJ 13355, 13356 or 13601. The activity of EDA and/or EDD is increased: (a) by increasing the copy number of the gene, by transformation with recombinant DNA; or (b) by modifying regulatory regions, especially use of a stronger promoter, so that gene expression is increased. Optionally: (a) activity of other genes involved in biosynthesis of (I) is also increased, especially citrate synthase, phosphoenolpyruvate carboxylase and glutamate dehydrogenase; or (b) activity of enzymes that divert intermediates away from synthesis of (I) are down regulated, especially alpha-ketoglutarate dehydrogenase or isocitrate lyase, particularly by mutation or gene disruption by homologous recombination. Preferred Process: This is production of L-Glu or of (I) that are produced by biosynthetic pathways in which Glu is an intermediate (Arg, Gln, Pro, Leu, Ile, Val or Ala). The modified bacteria are cultured at 20-45degreesC and pH 5-9.

USE - The method is specifically used to produce L-glutamic acid. ADVANTAGE - Increasing activity of the specified enzymes reduces

flow of carbon into the pentose phosphate pathway and increases that into the Entner-Doudoroff pathway, so increases the production of (I) that are derived from pyruvate intermediate.

EXAMPLE - The strain Enterobacter agglomerans AJ13601: (a) has a disrupted gene for alpha-ketoglutarate dehydrogenase; and (b) includes a plasmid containing citrate synthase (CS), phosphoenolpyruvate carboxylase and glutamate dehydrogenase genes. It was transformed with (a) pMV-CB(asterisk) containing the promoter of the Escherichia coli CS gene and the coding region of the Brevibacterium lactofermentum CS gene or (b) pMV-CB(asterisk).ED, as (a) but including the E. coli genes for 6-phoshogluconate dehydratase (EDD) and/or 2-keto-3-deoxy-6phosphogluconate aldolase (EDA). Both transformants were grown on nutrient medium of pH 6. In (a) L-glutamic acid yield was 30.4 g/l after 12 hours, and in (b) it was 36.8 g/l after 16 hours.(18 pages)

ACCESSION NUMBER: 2004-00510 BIOTECHDS

TITLE:

Production of L-amino acids in Gram-negative

bacteria, useful particularly for making glutamic acid, by

increasing activity of Entner-Doudoroff

pathway enzymes;

involving Enterobacter agglomerans culture medium

HARA Y; IZUI H; ASANO T; WATANABE Y; NAKAMATSU T AUTHOR:

PATENT ASSIGNEE: AJINOMOTO CO INC

PATENT INFO:

EP 1352966 15 Oct 2003

APPLICATION INFO: EP 2003-6936 26 Mar 2003

PRIORITY INFO: JP 2002-88668 27 Mar 2002; JP 2002-88668 27 Mar 2002

DOCUMENT TYPE:

Patent

LANGUAGE:

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1213 S L-AMINO ACID AND PRODUCTION

3 S L2 AND L1 1.3

=> s 12 and methanol

18 L2 AND METHANOL

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ANSWER 1 OF 18 MEDLINE on STN T<sub>1</sub>4

ΤI Compared D-amino acid oxidase expression in different Pichia pastoris host

AB To compare the DAAO expression level in different Pichia pastoris host strains, the gene encoding DAAO from Trigonopsis variabilis was cloned into plasmid pPIC3.5k and then transformed into P. pastoris GS115 and KM71 respectively. The positive transformants PDK13 (MutS) and PD27 (Mut+) were obtained by PCR analysis. Their optimal and different expression conditions were investigated. To compare with PD27, PDK13 was determined to poss a slower consumption of methanol, a longer induction time, a lower oxygen request and apparently higher expression of DAAO. The highest expression levels were reached up to 2700, 2500 IU/L in shaking flask and 10140, 8463.5 IU/L in fermentor respectively. The over-expression of DAAO can meet its large demand for production of 7-ACA, alpha-keto acid and L-amino acid.

In addition, the phenylpyruvate and L-phenylalanine were obtained by crude DAAO reacting with DL-phenylalanine at 37 degrees C for 3h.

ACCESSION NUMBER: 2005320159 IN-PROCESS

DOCUMENT NUMBER: PubMed ID: 15968991

TITLE: Compared D-amino acid oxidase expression in different

Pichia pastoris host strains.

AUTHOR: Feng Mei-Qing; Huang Hai; Shi Xun-Long; Yu Zhi-Liang; Yuan

Zhong-Yi; Zhou Pei

CORPORATE SOURCE: Fudan University, Shanghai 200032, China.

SOURCE: Sheng wu gong cheng xue bao = Chinese journal of

biotechnology, (2004 Jul) 20 (4) 572-7. Journal code: 9426463. ISSN: 1000-3061.

PUB. COUNTRY: China

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: Chinese

FILE SEGMENT: NONMEDLINE; IN-PROCESS; NONINDEXED; Priority Journals

ENTRY DATE: Entered STN: 20050623

Last Updated on STN: 20051215

L4 ANSWER 2 OF 18 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

TI **Production** of a sol-gel layer on a substrate useful for preparing devices e.g. biosensor involves neutralizing an acidified sol suspension and electro-depositing it over an electrically conductive surface.

AN 2004-468317 [44] WPIDS

AB WO2004048603 A UPAB: 20040712

NOVELTY - **Production** (P1) of a sol-gel layer on a substrate, comprising at least partially neutralizing an acidified sol suspension, contacting an electrically conductive surface (s) with the neutralized suspension, and applying an electrical potential to (s) to form a layer of sol-gel on it, is new.

 ${\tt DETAILED}$  <code>DESCRIPTION</code> - <code>INDEPENDENT</code> <code>CLAIMS</code> are included for the following:

- (1) producing (P2) a biological assay device such as a microassay or biosensor, comprising electrodepositing a layer of sol-gel containing a biological material onto a substrate; and
- (2) a biological assay device comprising (s) and a sol-gel containing at least one biological material.

USE - For producing a biological assay device (e.g. a biosensor or microarray) comprising 2 electrodes to detect analytes (claimed).

ADVANTAGE - The method allows microarrays and biosensors to be made inexpensively. The biosensors prepared by using this method have increased sensitivity (100-fold) than those prepared in prior arts. Stock solutions of acidified sol can be stored for long time. The enzymes may be added as and when a specific electrode needs to be produced. This allows greater flexibility in the **production** of microarrays and biosensors.

Dwg.0/3

ACCESSION NUMBER: 2004-468317 [44] WPIDS

DOC. NO. NON-CPI: N2004-370030 DOC. NO. CPI: C2004-175494

TITLE: Production of a sol-gel layer on a substrate

useful for preparing devices e.g. biosensor involves

neutralizing an acidified sol suspension and

electro-depositing it over an electrically conductive

surface.

DERWENT CLASS: A89 B04 D16 E11 J04 S03

INVENTOR(S): DALE, N E; DRONIOU, M; LLAUDET, E

PATENT ASSIGNEE(S): (UYWA-N) UNIV WARWICK

COUNTRY COUNT: 108

PATENT INFORMATION:

PATENT NO KIND DATE WEEK LA PG
WO 2004048603 A2 20040610 (200444)\* EN 29

RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE

LS LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ

VC VN YU ZA ZM ZW

A1 20040618 (200471) AU 2003283590

EP 1565565 A2 20050824 (200556) EN

R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LT LU LV MC MK NL PT RO SE SI SK TR

#### APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2004048603	A2	WO 2003-GB4984	20031118
AU 2003283590	A1	AU 2003-283590	20031118
EP 1565565	A2	EP 2003-775567	20031118
		WO 2003-GB4984	20031118

#### FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2003283590	A1 Based on	WO 2004048603
EP 1565565	A2 Based on	WO 2004048603

PRIORITY APPLN. INFO: GB 2002-27424 20021125

- ANSWER 3 OF 18 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
- ΤI Enzymatic peptide synthesis, by contacting two amino acids, one peptide and one amino acid, or two peptides, with a peptide ligase to form a dipeptide, and contacting the dipeptide with a deacetylase to form peptide.
- AN 2003-569047 [53] WPIDS
- WO2003048189 A UPAB: 20030820 AΒ

NOVELTY - Enzymatic peptide synthesis (M), comprising contacting two amino acids, one peptide and one amino acid, or two peptides, with a peptide ligase (PL) so that PL catalyzes formation of peptide bond between the amino acids or between the peptide and the amino acid, or between the peptides, thus making a dipeptide (DP), and contacting DP with a deacetylase (DA) so that DA catalyzes deacylation of DP, is new.

DETAILED DESCRIPTION - An enzymatic process (M) for synthesizing a peptide involves:

- (a) providing at least two amino acids, at least one peptide and at least one amino acid, or at least two peptides;
- (b) providing a peptide ligase and a deacetylase, where the peptide ligase and the deacetylase are active under different reaction conditions and the peptide ligase is active after exposure to reaction conditions where the deacetylase is active, and the deacetylase is active after exposure to reaction conditions where the peptide ligase is active;
- (c) contacting the amino acids, or the peptide and amino acid, or peptides, with the peptide ligase under conditions, where the peptide ligase catalyzes the formation of a peptide bond between the amino acids or between the peptide and the amino acid, or between the peptides, thus making at least a dipeptide; and
- (d) contacting the peptide with the deacetylase under conditions, where the deacetylase catalyzes the deacylation of the peptide, to synthesize a peptide or a polypeptide.

Alternatively, the method comprises:

(a) providing reaction chamber comprising a peptide ligase and a deacetylase, where the peptide ligase and deacetylase are active under different reaction conditions, and the peptide ligase is active after

exposure to reaction conditions where the deacetylase is active, and the deacetylase is active after exposure to reaction conditions where the peptide ligase is active;

- (b) adding at least two amino acids, at least an amino acid and a peptide, or at least two peptides to the reaction chamber under conditions, where the peptide ligase is active and the peptide ligase catalyzes the formation of a peptide bond between the amino acids, or between the peptide and the amino acid, or between the peptides; and
- (c) changing the conditions in the reaction chamber to conditions, where the deacetylase is active and the deacetylase catalyzes the deacylation of the peptide formed.

An INDEPENDENT CLAIM is also included for a product of manufacture (I) comprising a reaction chamber for synthesizing a peptide comprising a peptide ligase and a deacetylase, where the peptide ligase and the deacetylase are active under different reaction conditions and the peptide ligase is active after exposure to reaction conditions, and the deacetylase is active after exposure to reaction conditions where the peptide ligase is active.

USE - (M) is useful for synthesizing a peptide or polypeptide, where the peptide or polypeptide is between 2-50, preferably 5-25 peptides in length. The peptide is a D-amino acid, a naturally occurring Lamino acid, or a glycosylated, phosphorylated or non-naturally occurring amino acid, where the non-naturally occurring amino acid is a non-naturally occurring aromatic amino acid comprising a D- or L-naphthylalanine, D- or L-phenylglycine, D- or L-2 thieneylalanine, D- or L-1, -2, 3- or 4-pyreneylalanine, D- or L-3 thienylalanine, D- or L-(2-pyridinyl)-alanine, D- or L-(3-pyridinyl)-alanine, a D- or L-(2-pyrazinyl)-alanine, D- or L-(3-pyridinyl)-alanine, D- or L-(2-pyrazinyl)-alanine, D- or L-(4-isopropyl)-phenylglycine, a D-(trifluoromethyl)-phenylglycine, a D-(trifluoromethyl)-phenylalanine, a D-p-fluoro-phenylalanine, a D-or L-p-biphenylphenylalanine, a D-or L-p-methoxy-biphenylphenylalanine, D- or L-2-indole(alkyl)alanines. The non-naturally occurring aromatic amino acid comprises a thiazolyl, a thiophenyl, a pyrazolyl, a benzimidazolyl, a naphthyl, a furanyl, pyrrolyl, or pyridyl aromatic ring. The non-naturally occurring amino acid comprises a D- or L-alkylalanine. The alkyl of the alkylalanines comprises a substituted or unsubstituted methyl, ethyl, propyl, hexyl, butyl, pentyl, isopropyl, iso-butyl, sec-isotyl or iso-pentyl (claimed). (I) is useful for synthesizing commercial drugs such as Lupron (RTM), Leuplin (RTM), Miacalcin (RTM), Zoladex (RTM), Sandostatin (RTM), Decapeptyl (RTM), DDAVP (RTM), Glucagon (RTM), Intergrilin (RTM), Suprefact (RTM), Stilamin (RTM), Synarel (RTM), Zadaxin (RTM) or Copaxone (RTM).

ADVANTAGE - (M) uses mild conditions for coupling and deprotection steps, has a reduced requirement for organic solvents, avoids expensive and toxic coupling reagents, reduces the **production** of toxic byproducts, and allows for biocatalyst recycling. Dwq.0/6

ACCESSION NUMBER: 2003-569047 [53] WPIDS

DOC. NO. CPI: C2003-153465

TITLE: Enzymatic peptide synthesis, by contacting two amino acids, one peptide and one amino acid, or two peptides, with a peptide ligase to form a dipeptide, and contacting

the dipeptide with a deacetylase to form peptide.

DERWENT CLASS: B04 D16

INVENTOR(S): BURK, M J; DESANTIS, G; BURK, M; DE SANTIS, G

PATENT ASSIGNEE(S): (DIVE-N) DIVERSA CORP

COUNTRY COUNT: 102

PATENT INFORMATION:

RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU

MC MW MZ NL OA PT SD SE SI SK SL SZ TR TZ UG ZM ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW

US 2003143664 A1 20030731 (200354) AU 2002364710 A1 20030617 (200419)

#### APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2003048189	A2	WO 2002-US38588	20021203
US 2003143664	Al Provisional	US 2001-336580P	20011203
		US 2002-309758	20021203
AU 2002364710	A1	AU 2002-364710	20021203

#### FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2002364710	Al Based on	WO 2003048189

PRIORITY APPLN. INFO: US 2001-336580P 20011203; US 2002-309758 20021203

- L4 ANSWER 4 OF 18 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
- TI Efficient production of L-amino-acids e.g. L-lysine by fermenting transformed Methylophilus bacterium with enhanced dihydrodipicolinate synthase and aspartokinase activities or casamino acid requirement using methanol.
- AN 2000-672679 [65] WPIDS
- AB WO 200061723 A UPAB: 20001214

NOVELTY - A bacterium of Methylophilus genus can produce an **L**-amino-acid efficiently, which is a transformed bacterium with enhanced enzyme activity.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

- (1) a process for preparing an L-aminoacid by culturing Methylophilus for accumulation of the product in culture medium before isolation;
- (2) a DNA having a 1981 base pair (bp) sequence, encoding a 409 amino acid sequence, having aspartokinase activity;
- (3) a DNA having a 1452 bp sequence, encoding a 370 amino acid sequence, aspartic semi-aldehyde dehydrogenase activity;
- (4) a DNA having a 3098 bp sequence, encoding a 296 amino acid sequence, having dihydrodipicolinate synthase activity;
- (5) a DNA having a 3390 bp sequence, encoding the 286 amino acid sequence, dihydrodipicolinate reductase activity; and
- (6) a DNA having the 2566 bp sequence, encoding the 415 amino acid sequence, having diaminopimelate decarboxylate activity.

All sequences are given in the specification.

USE - The method is for the **production** of L-amino-acids including L-lysine, L-valine, L-leucine, L-isoleucine or L-threonine (claimed).

ADVANTAGE - The process is efficient and the bacterium used has enhanced enzyme activity..

 $\label{eq:def:DESCRIPTION OF DRAWING(S) - Construction of mutated dap A-containing \\ plasmid RSF24P.$ 

Dwq.1/7

ACCESSION NUMBER: 2000-672679 [65] WPIDS DOC. NO. CPI: C2000-203771

Efficient production of L-amino-acids e.g. TITLE:

L-lysine by fermenting transformed Methylophilus

bacterium with enhanced dihydrodipicolinate synthase and aspartokinase activities or casamino acid requirement

using methanol.

B05 D16 E19 DERWENT CLASS:

GUNJI, Y; MIYATA, Y; OBA, M; SHIMAOKA, M; SUGIMOTO, S; INVENTOR(S):

TSUJIMOTO, N; YASUEDA, H

PATENT ASSIGNEE(S): (AJIN) AJINOMOTO CO INC

COUNTRY COUNT: 35

PATENT INFORMATION:

PATENT NO	KIND DATE	WEEK LA	A PG
WO 2000061723	A1 20001019	(200065)* JA	92
RW: AT BE CH	CY DE DK ES	FI FR GB GR IE	E IT LU MC NL PT SE
W: AU BR CA	CN CZ HU ID	JP KR MX PL RU	JSK US VN ZA
AU 2000036745	A 20001114	(200108)	
BR 2000009550	A 20020205	(200213)	
EP 1188822	A1 20020320	(200227) EN	
R: AT BE CH	CY DE DK ES	FI FR GB GR IE	E IT LI LU MC NL PT SE
CN 1346402	A 20020424	(200251)	
JP 2000611648	X 20020723	(200263)	
MX 2001010151	A1 20030701	(200366)	
RU 2250266	C2 20050420	(200528)	

#### APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000061723	A1	WO 2000-JP229	20000407
AU 2000036745	Α	AU 2000-36745	20000407
BR 2000009550	A	BR 2000-9550	20000407
		WO 2000-JP229	20000407
EP 1188822	A1	EP 2000-91543	6 20000407
		WO 2000-JP229	20000407
CN 1346402	A	CN 2000-80601	.9 20000407
JP 2000611648	X	JP 2000-61164	8 20000407
		WO 2000-JP229	5 20000407
MX 2001010151	A1	WO 2000-JP229	5 20000407
		MX 2001-10151	20011008
RU 2250266	C2	WO 2000-JP229	5 20000407
		RU 2001-13014	6 20000407

#### FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2000036745 BR 2000009550	A Based on A Based on	WO 2000061723 WO 2000061723
EP 1188822	Al Based on	WO 2000061723
JP 2000611648 MX 2001010151	X Based on Al Based on	WO 2000061723 WO 2000061723
RU 2250266	C2 Based on	WO 2000061723

JP 1999-368097 19991224; 1999-103143 19990409; JP 1999-169447 19990616 PRIORITY APPLN. INFO: JP 1999-368097 19991224; JP

L4 ANSWER 5 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN Novel modified microorganism capable of showing enhanced expression of TI ybjE gene and producing L-amino acid such as L-lysine, L-arginine, L-ornithine, useful as additives for animal

feed, amino acid infusions and precursor of sweeteners; amino acid production via genetically engineered bacterium culture for use in food 2005-25581 BIOTECHDS ΑN DERWENT ABSTRACT: AB NOVELTY - A microorganism (I) having an L-amino acid-producing ability, where the microorganism is modified so that expression of ybjE gene is enhanced, is new. BIOTECHNOLOGY - Preferred Microorganism: In (I), the expression of the ybjE gene has been enhanced by increasing a copy number of the ybjE gene, or by modifying an expression regulatory sequence of ybjE gene. The amino acid sequence of a protein encoded by ybjE gene is chosen from fully defined 315 amino acid (SEQ ID NO: 2), 299 amino acid (SEQ ID No: 9) and 298 amino acid (SEQ ID No: 10) sequences given in the specification, where the protein has an L-amino acid-export ability. The ybjE gene is chosen from a DNA comprising a fully defined 948 nucleotides (SEQ ID NO: 1) sequences given in the specification, and a DNA hybridizable under stringent conditions with a nucleotide sequence of SEQ ID No: 1 or a probe that can be prepared from the nucleotide sequence of SEQ ID No: 1, where the DNA encodes a protein having an L-amino acid -export ability. The ybjE gene is chosen from a DNA having a nucleotide sequence of nucleotide numbers 49-948 in SEQ ID No: 1, and a DNA hybridizable under stringent conditions with a nucleotide sequence of nucleotide numbers 49-948 in SEQ ID No: 1 or a probe that can be prepared from the nucleotide sequence of nucleotide numbers 49-948 in SEQ ID No: 1, and where the DNA encodes a protein having an  $\boldsymbol{\mathtt{L}}\text{-}$ amino acid-export ability. The Lamino acid-export ability of the microorganism is increased by enhancing expression of ybjE gene. The resistance of the microorganism to an L-amino acid or L-amino acid analog is increased by enhancing expression of the ybjE gene. The microorganism belongs to an Enterobacteriaceae family. The microorganism belonging to an Enterobacteriaceae family is a microorganism belonging to the genus Escherichia. The microorganism is a Coryneform bacterium or a methanol-assimilating bacterium. The methanol -assimilating bacterium is a microorganism belonging to the genus Methylophilus or Methylobacillus. USE - (I) is useful for producing L-amino acid, which involves culturing (I) in a medium to produce and cause accumulation of the L-amino acid, and collecting the L-amino acid from the medium or (I), or by culturing (I) in a liquid containing methanol as a major carbon source to produce and cause the accumulation of  ${f L}$ -amino acid and collecting L-amino acid from (I). The L-amino acid is chosen from L-lysine, L-arginine, L-ornithine, L-histidine, L-isoleucine, L-threonine, L-proline, L-phenylalanine, L-cysteine, and L-glutamic acid (claimed). The L-lysine, L-isoleucine, L-threonine, L-proline are useful as additives for animal feed, components of health food and amino acid infusions. The L-arginine and L-ornithine are useful as liver function-promoting agents, amino acid infusions and components of comprehensive amino acid preparations. The L-histidine is useful as a liver function-promoting agent and as a precursor of histamine. The L-phenylalanine is useful as a precursor of sweeteners. ADVANTAGE - (M1) is cost effective and efficient, and introduction

EXAMPLE - The AS1 strains introduced with pRSybjEdapA, pRSybjE, pRSdaPA, or pRS, were plated on SEII plate containing 20 mg/l of streptomycin and cultured overnight at 37degreesC. Then, the cells from 0.3 cm2 of the medium surface were scraped, inoculated in to SEII

of ybjE gene increases L-amino acid export

ability.

production medium (20 ml) containing streptomycin (20 mg/l), and cultured at 37degreesC for 34 hours with shaking. After completion of the culture, the cells were removed by centrifugation, and the L-1ysine concentration in the culture supernatant was determined by using an amino acid analyzer. The strain that had been introduced with pRSybjEdapA showed increased L-lysine accumulation (1.38 g/l) compared to the strains introduced only with pRSdapA (0.12 g/l) or pRsybje (0.7 g/l), and pRS (0 g/1) . Thus, it was found that enhancing both the ybjE gene and the dapAasterisk gene had a synergistic effect on L-lysine production . (85 pages)

ACCESSION NUMBER: 2005-25581 BIOTECHDS

TITLE:

AN

Novel modified microorganism capable of showing enhanced

expression of ybjE gene and producing Lamino acid such as L-lysine, L-arginine,

L-ornithine, useful as additives for animal feed, amino acid

infusions and precursor of sweeteners;

amino acid production via genetically engineered

bacterium culture for use in food

AUTHOR:

UEDA T; NAKAI Y; GUNJI Y; TAKIKAWA R; JOE Y

PATENT ASSIGNEE: AJINOMOTO CO INC

PATENT INFO: WO 2005073390 11 Aug 2005 APPLICATION INFO: WO 2005-JP1650 28 Jan 2005

PRIORITY INFO: JP 2004-23347 30 Jan 2004; JP 2004-23347 30 Jan 2004 DOCUMENT TYPE: Patent

DOCUMENT TYPE: English LANGUAGE:

OTHER SOURCE: WPI: 2005-604908 [62]

ANSWER 6 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN ΤI Production of target substance, e.g. L-amino acids, using coryneform bacterium, comprises culturing coryneform bacterium having ability to produce target substance in medium, and collecting target substance from medium or cells of bacterium;

L-amino acid production via

modified bacterium culture

2004-21654 BIOTECHDS

AΒ DERWENT ABSTRACT:

NOVELTY - Target substance, e.g. L-amino acids, is produced using coryneform bacterium by culturing a coryneform bacterium having an ability to produce the target substance in a medium to result in accumulation of the target substance in the medium or cells of the bacterium, and collecting the target substance from the medium or cells of bacterium.

DETAILED DESCRIPTION - Production of target substance, e.g. L-amino acids, using coryneform bacterium comprises culturing a coryneform bacterium having an ability to produce the target substance in a medium to result in accumulation of the target substance in the medium or cells of the bacterium, and collecting the target substance from the medium or cells of bacterium. A methanol dehydrogenase, hexulose phosphate synthase gene, and phosphohexuloisomerase gene are introduced into the coryneform bacterium. The bacterium is modified so that an ability to utilize methanol is imparted. It contains methanol as carbon source.

BIOTECHNOLOGY - Preferred Process: The bacterium is further introduced with a gene encoding a methanol dehydrogenase activity-promoting factor. Preferred Component: The bacterium belongs to the genus corynebacterium, preferably Corynebacterium glutamicum.

USE - For producing a target substance, e.g. L-amino acids from L-lysine (claimed), nucleic acids, antibiotics, vitamins, growth factors, and physiologically actives substances, using coryneform bacterium.

ADVANTAGE - The invention enhances the ability of the bacterium to utilize methanol. (28 pages)

ACCESSION NUMBER: 2004-21654 BIOTECHDS

TITLE:

Production of target substance, e.g. L-amino acids,

using coryneform bacterium, comprises culturing coryneform bacterium having ability to produce target substance in medium, and collecting target substance from medium or cells of bacterium;

#### L-amino acid

production via modified bacterium culture

AUTHOR: TAKESHITA R; YASUEDA H

PATENT ASSIGNEE: AJINOMOTO CO INC

PATENT INFO: EP 1454991 8 Sep 2004 APPLICATION INFO: EP 2004-5129 4 Mar 2004

PRIORITY INFO: JP 2003-57171 4 Mar 2003; JP 2003-57171 4 Mar 2003

DOCUMENT TYPE: Patent LANGUAGE: English

OTHER SOURCE: WPI: 2004-627851 [61]

L4 ANSWER 7 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN L-Lysine preparation with high productivity, by culturing methanol-utilizing bacteria auxotrophic for L-methionine,

preferably new Methylophilus strains;

L-amino acid preparation by bacterium

fermentation

AN 2004-20239 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - **Production** of L-lysine (I) involves culturing bacteria which utilize **methanol**, require L-methionine (II) for growth and produce (I) in a medium containing **methanol** as main carbon source, then recovering the (I) accumulated in the culture, is new.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is included for Methylophilus bacteria which require (II) for growth and produce (I).

BIOTECHNOLOGY - Preferred Bacteria: The bacteria are of genus Methylophilus, preferably Methylophilus methylotrophus strains, especially Methylophilus strains modified to stimulate diaminopimelate enzymatic activity and the (I) secretion system. Production:
The new Methylophilus bacteria requiring (II) for growth are obtained by mutation of wild strains using physical stimuli (e.g. UV-, X- or gamma-rays) or preferably a chemical mutagen (e.g. N-methyl-N'-nitro-N-nitroso-guanidine); or by using genetic engineering methods to suppress the activity of an enzyme involved in the synthesis of (II), e.g. by cleaving the gene metA (encoding homoserine o-acetyltransferase) in

USE - For producing L-lysine (claimed).

Methylophilus methylotrophus.

ADVANTAGE - (I) are obtained with high efficacy from bacteria utilizing (inexpensive) methanol as carbon source. Conferring auxotrophy for (II) on methanol-utilizing bacteria significantly increases the amount of free (I) accumulated in the cells relative to the total amount of cell proteins, e.g. by a factor of 1.5 or more.

EXAMPLE - The wild strain Methylophilus methylotrophus AS1 (NCIMB 10515) was subjected to cleavage of the gene metA (encoding homoserine o-acetyltransferase). A DNA region of high homology with this gene (cleaved from Mycobacterium tuberculosis strain H37Rv (CAA17113)), chromosomal DNA from strain AS1 and plasmid pKD4 (encoding kanamycin resistance) were separately amplified by PCR, mixed and subjected to further PCR to give a 4.2 kb fragment, consisting of gene metA with the kanamycin resistance gene inserted. This fragment was introduced into strain AS1 by electroporation to give a strain auxotrophic for L-methionine (II), designated strain MR701. The L-lysine (I)-producing plasmid pSEA10 was introduced into strain MR701 by electroporation to give the (I)-producing strain MR701(pSEA10). Cells of this strain were used to inoculate production medium SEII containing 0.075 g/l (II), followed by culture under stirring at 37degreesC for 48 hours. The amount of (I) accumulated in the supernatant was 1.52 g/l, compared with

0.97 g/l using the strain AS1(pSEA12).(43 pages)

ACCESSION NUMBER: 2004-20239 BIOTECHDS

L-Lysine preparation with high productivity, by culturing TITLE:

methanol-utilizing bacteria auxotrophic for

L-methionine, preferably new Methylophilus strains;

L-amino acid preparation by

bacterium fermentation

PATENT ASSIGNEE: AJINOMOTO CO INC

PATENT INFO: FR 2850394 30 Jul 2004 APPLICATION INFO: FR 2004-816 29 Jan 2004

PRIORITY INFO: JP 2003-20513 29 Jan 2003; JP 2003-20513 29 Jan 2003

DOCUMENT TYPE: Patent LANGUAGE:

AB

French

OTHER SOURCE:

WPI: 2004-563572 [55]

ANSWER 8 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

TI Production of an L-amino acid

> using an amino acid, an ammonium salt, a reducing coenzyme, and a microorganism with transaminase and amino acid dehydrogenase activity, to convert an alpha-keto acid into an L-amino acid;

L-t-leucine preparation by bacterium fermentation and enzyme-catalyzed conversion

ΔN 2004-11885 BIOTECHDS

DERWENT ABSTRACT:

NOVELTY - Production (M1) of an L-amino

acid (X) using an amino acid as an amino donor, an ammonium salt and coenzyme which has reducing power, and converting an alpha-keto acid or its salt into L-amino acids in the presence of a microorganism (or its product) which has transaminase activity and amino acid dehydrogenase activity.

BIOTECHNOLOGY - Preferred Method: The co-enzyme is reduced nicotinamide adenine dinucleotide, reduced nicotinamide adenine dinucleotide phosphoric acid or their salts. Amino acid (X) is a compound of formula (I). R = alkyl group of two or more carbon atoms, a benzyl group, a p-hydroxy benzyl group or an L-amino acid. Preferred Microorganism: The microorganism providing the enzyme activity is preferably Brevibacterium ammoniagenes.

USE - Process M1 is useful for producing (X) such as L-t-leucine from alpha-keto acids (claimed).

ADVANTAGE - Process M1 enables production of large amounts of L-amino acid.

EXAMPLE - Microbial cells e.g. Brevibacterium ammoniagenes ATCC 6872, with transaminase activity and amino acid dehydrogenase activity, were inoculated into culture media comprising glucose, dipotassium hydrogen phosphate, magnesium sulfate, peptone, sodium chloride and yeast extract, and cultivated by shaking at 37 degrees C until the absorbance of 600 nm was 0.7. The cultivated microbial cells were collected and washed by 0.85% sodium chloride and then allowed to dry overnight. Dry microbial cells (10 mg/ml) were added to reaction solution (A) which contained 60 q/l of glutamic acid with respect to 40 q/l of trimethyl pyruvic acid, reaction solution (B) which contained 40 g/l of ammonium chloride, 100 g/l of glucose, 1.2 g/l of nicotinamide adenine dinucleotide (NADH), 1.2 g/l of nicotinamide adenine dinucleotide phosphoric acid (NADPH) and 0.41 g/l of glucose dehydrogenase, and reaction solution (C) which contained 60 g/l of glutamic acid, 40 g/l of ammonium chloride, 100 g/l of glucose, 1.2 g of NADH, 1.2 g of NADPH and 0.41 g/l of glucose dehydrogenase. The solutions were allowed to react at 30 degrees C for six days. To 100 microl of obtained reaction solution, 10 mM gamma amino butyric acid was added and extracted by 200 microl of 1-butanol. To 10 microl of obtained solution, 1% trimethylamine aqueous solution, 20 microl of acetonitrile and 0.2% of 50 microl of 2,3,4,6-tetra-o-acetyl-beta-D-glucoisothiocyanate (GITC)-acetonitrile

were added and allowed to react for 15 minutes at room temperature with respect to 20 microl of 1-butanol layers. 5 microl of the obtained solution was subjected to high performance layer chromatography which uses shim-pack CLC-ODS(M) column, and 60% aqueous solution of methanol as eluting solvent for analysis of L-t-leucine in a sample. The concentration of L-t-leucine in a sample was calculated based on the standard curve. The results showed that reaction solution (C) which had both the transaminase activity and amino acid dehydrogenase activity produced more amount of L-t-leucine when compared to reaction solution (A) having transaminase activity and reaction solution (B) having amino acid dehydrogenase activity. The concentration of L-t-leucine produced by using reaction solution (C) was found to be 3.4 g/l. (6 pages)

ACCESSION NUMBER: 2004-11885 BIOTECHDS

TITLE:

ΑN

Production of an L-amino

acid using an amino acid, an ammonium salt, a
reducing coenzyme, and a microorganism with transaminase and
amino acid dehydrogenase activity, to convert an alpha-keto
acid into an L-amino acid;

L-t-leucine preparation by bacterium fermentation and

enzyme-catalyzed conversion

PATENT ASSIGNEE: TORAY IND INC

PATENT INFO: JP 2003284584 7 Oct 2003 APPLICATION INFO: JP 2002-94838 29 Mar 2002

PRIORITY INFO: JP 2002-94838 29 Mar 2002; JP 2002-94838 29 Mar 2002 DOCUMENT TYPE: Patent

LANGUAGE: Japanese

OTHER SOURCE: WPI: 2004-172965 [17]

ANSWER 9 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
Enzymatic peptide synthesis, by contacting two amino acids, one peptide
and one amino acid, or two peptides, with a peptide ligase to form a
dipeptide, and contacting the dipeptide with a deacetylase to form
peptide;

enzyme-catalyzed peptide synthesis for use in the drug industry 2003-20758 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - Enzymatic peptide synthesis (M), comprising contacting two amino acids, one peptide and one amino acid, or two peptides, with a peptide ligase (PL) so that PL catalyzes formation of peptide bond between the amino acids or between the peptide and the amino acid, or between the peptides, thus making a dipeptide (DP), and contacting DP with a deacetylase (DA) so that DA catalyzes deacylation of DP, is new.

DETAILED DESCRIPTION - An enzymatic process (M) for synthesizing a peptide involves: (a) providing at least two amino acids, at least one peptide and at least one amino acid, or at least two peptides; (b) providing a peptide ligase and a deacetylase, where the peptide ligase and the deacetylase are active under different reaction conditions and the peptide ligase is active after exposure to reaction conditions where the deacetylase is active, and the deacetylase is active after exposure to reaction conditions where the peptide ligase is active; (c) contacting the amino acids, or the peptide and amino acid, or peptides, with the peptide ligase under conditions, where the peptide ligase catalyzes the formation of a peptide bond between the amino acids or between the peptide and the amino acid, or between the peptides, thus making at least a dipeptide; and (d) contacting the peptide with the deacetylase under conditions, where the deacetylase catalyzes the deacylation of the peptide, to synthesize a peptide or a polypeptide. Alternatively, the method comprises: (a) providing reaction chamber comprising a peptide ligase and a deacetylase, where the peptide ligase and deacetylase are active under different reaction conditions, and the peptide ligase is active after exposure to reaction conditions where the deacetylase is active, and the deacetylase is active after exposure to reaction

conditions where the peptide ligase is active; (b) adding at least two amino acids, at least an amino acid and a peptide, or at least two peptides to the reaction chamber under conditions, where the peptide ligase is active and the peptide ligase catalyzes the formation of a peptide bond between the amino acids, or between the peptide and the amino acid, or between the peptides; and (c) changing the conditions in the reaction chamber to conditions, where the deacetylase is active and the deacetylase catalyzes the deacylation of the peptide formed. An INDEPENDENT CLAIM is also included for a product of manufacture (I) comprising a reaction chamber for synthesizing a peptide comprising a peptide ligase and a deacetylase, where the peptide ligase and the deacetylase is active after exposure to reaction conditions, and the deacetylase is active after exposure to reaction conditions where the peptide ligase is active after exposure to reaction conditions where the peptide ligase is active after exposure to reaction conditions where the peptide ligase is active.

BIOTECHNOLOGY - Preferred Method: (M) further comprises changing conditions in the reaction chamber to conditions, where the peptide ligase is active and the deacetylase is inactive and adding at least one additional amino acid or peptide to the reaction chamber. (M) further comprises changing conditions in the reaction chamber to conditions so that the deacetylase is active. The temperature conditions are changed in the reaction chamber. (M) further comprises reiterating the process, thus making a longer peptide or polypeptide. The peptide ligase and the deacetylase are active at different temperature conditions. The reiterated process comprises thermocycling the peptide ligase and the deacylation reactions. The reaction chamber comprises a thermocycled bioreactor. The peptide ligase and the deacetylase are active at different pH conditions, at different salt conditions or at different solute conditions. The reaction chamber comprises a capillary array such as GIGAMATRIX (RTM). The peptide ligase and the deacetylase are immobilized. The peptide ligase is active at higher temperature than the deacetylase. The deacetylase is inactive and thermotolerant in the conditions set for the peptide ligase activity. The amino acids, or the peptide and the amino acid, are contacted with the peptide ligase under conditions comprising about a temperature of 50 degreesC. The deacylation reaction conditions comprise a temperature of 20 degreesC. The peptide ligase and the deacetylase reactions are thermocycled between 50-20 degreesC. The peptide ligase is a hydrolase (e.g. serine hydrolase), esterase or lipase, muramyl peptide synthetase, or a catalytic antibody. The deacetylase is an aminoacylase, D-aminoacylase, L-aminoacylase or a catalytic antibody. (M) further comprises use of at least two peptide ligases or at least two aminoacylases. The reaction is driven in favor of peptide catalysis and reducing aminolysis by reacting the peptide ligase under conditions comprising a low water environment, an organic solvent, or substantially pure organic solvent, or under conditions comprising removing the product upon its formation by precipitation or by liquid-liquid extraction. The reaction is driven in favor of peptide catalysis and reducing aminolysis by reacting the peptide ligase in a water and ethanol solvent, or in a pure methanol solvent or a pure ethanol solvent. (M) further comprises the injection of fresh enzyme into the reaction after each reiterated cycle. (M) further comprises the injection of fresh peptide ligase or deacetylase into the reaction. (M) comprises providing reaction chamber comprising a peptide ligase and a deacetylase, where the peptide ligase and deacetylase are active at different temperatures and the peptide ligase is active after exposure to the deacetylase's activity temperature and the deacetylase is active after exposure to the peptide ligase's activity temperature, and at least two amino acids, at least an amino acid and a peptide, or at least two peptides, reacting the reaction chamber under conditions where the peptide ligase is active and the deacetylase is inactive and the peptide ligase catalyzes the formation of a peptide bond between the amino acids, or between the peptide and the amino acid, or between the peptides, and changing the conditions in the reaction chamber to conditions where the

deacetylase is active and the peptide ligase is inactive and the deacetylase catalyzes the deacylation of the peptide formed. Preferred Product: In (I), the reaction chamber comprises a thermocycler. The reaction chamber is operably linked to an high pressure liquid chromatograph, mass spectrograph, a liquid chromatograph (LC), or a multiplex interfaced liquid chromatograph (LC)-mass spectrograph (MS) (LC-MS) system. The reaction chamber further comprises a desorption/ionization device. (I) further comprises an input for injection of enzyme or starting material into the reaction chamber, robotic arms to move microtiter plates between different platform components, temperature and humidity controlled incubators, liquid handling devices, bar-coding devices or plate readers. (I) enables high throughput robotic assays.

USE - (M) is useful for synthesizing a peptide or polypeptide, where the peptide or polypeptide is between 2-50, preferably 5-25 peptides in length. The peptide is a D-amino acid, a naturally occurring Lamino acid, or a glycosylated, phosphorylated or non-naturally occurring amino acid, where the non-naturally occurring amino acid is a non-naturally occurring aromatic amino acid comprising a D- or L-naphthylalanine, D- or L-phenylglycine, D- or L-2 thieneylalanine, D- or L-1, -2, 3- or 4-pyreneylalanine, D- or L-3 thienylalanine, D- or L-(2-pyridinyl)-alanine, D- or L-(3-pyridinyl)alanine, a D- or L-(2-pyrazinyl)-alanine, D- or L-(3-pyridinyl)-alanine, D- or L-(2-pyrazinyl)-alanine, D- or L-(4-isopropyl)-phenylglycine, a D-(trifluoromethyl)-phenylglycine, a D-(trifluoromethyl)-phenylalanine, a D-p-fluoro-phenylalanine, a D-or L-p-biphenylphenylalanine, a D-or L-p-methoxy-biphenylphenylalanine, D- or L-2-indole(alkyl)alanines. The non-naturally occurring aromatic amino acid comprises a thiazolyl, a thiophenyl, a pyrazolyl, a benzimidazolyl, a naphthyl, a furanyl, pyrrolyl, or pyridyl aromatic ring. The non-naturally occurring amino acid comprises a D- or L-alkylalanine. The alkyl of the alkylalanines comprises a substituted or unsubstituted methyl, ethyl, propyl, hexyl, butyl, pentyl, isopropyl, iso-butyl, sec-isotyl or iso-pentyl (claimed). (I) is useful for synthesizing commercial drugs such as Lupron (RTM), Leuplin (RTM), Miacalcin (RTM), Zoladex (RTM), Sandostatin (RTM), Decapeptyl (RTM), DDAVP (RTM), Glucagon (RTM), Intergrilin (RTM), Suprefact (RTM), Stilamin (RTM), Synarel (RTM), Zadaxin (RTM) or Copaxone (RTM).

ADVANTAGE - (M) uses mild conditions for coupling and deprotection steps, has a reduced requirement for organic solvents, avoids expensive and toxic coupling reagents, reduces the **production** of toxic byproducts, and allows for biocatalyst recycling. (43 pages)

ACCESSION NUMBER: 2003-20758 BIOTECHDS

TITLE: Enzymatic peptide synthesis, by contacting two amino acids,

one peptide and one amino acid, or two peptides, with a peptide ligase to form a dipeptide, and contacting the

dipeptide with a deacetylase to form peptide;

enzyme-catalyzed peptide synthesis for use in the drug

industry

AUTHOR: DE SANTIS G; BURK M

PATENT ASSIGNEE: DIVERSA CORP

PATENT INFO: WO 2003048189 12 Jun 2003 APPLICATION INFO: WO 2002-US38588 3 Dec 2002

PRIORITY INFO: US 2001-336580 3 Dec 2001; US 2001-336580 3 Dec 2001

DOCUMENT TYPE: Patent LANGUAGE: English

OTHER SOURCE: WPI: 2003-569047 [53]

ANSWER 10 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

Novel DNA encoding variant of LysE protein from a coryneform bacterium, when introduced into methanol assimilating bacterium, facilitates excretion of L-lysine and/or L-arginine to outside of a cell; plasmid pRSlysE-mediated lysE gene transfer to Methylophilus sp. for

amino acid production

2003-11377 BIOTECHDS

DERWENT ABSTRACT:

AN AB

NOVELTY - DNA (I) encoding variant of protein with loop region and six hydrophobic helixes facilitates excretion of L-lysine and/or L-arginine to outside of cell of a **methanol** assimilating bacterium when (I) is introduced into the bacterium, is new.

DETAILED DESCRIPTION - DNA (I) encoding variant of protein with loop region and six hydrophobic helixes and involved in excretion of L-lysine to outside of cell, where (I) encodes a mutant protein not containing loop region that is contained in wild-type protein and facilitates excretion of L-lysine, L-arginine or both of these L-amino acids to outside of a cell of a methanol assimilating bacterium when (I) is introduced into the bacterium, is new. INDEPENDENT CLAIMS are also included for: (1) A DNA encoding: (a) a protein which comprises a fully defined sequence of 124 amino acids (S1) as given in the specification; or (b) a protein which comprises a sequence of (S1) including substitution, deletion, insertion or addition of one or several amino acid residues and shows an activity for facilitating excretion of L-lysine, L-arginine or both of these L-amino acids to outside of a cell of methanol assimilating bacterium; (2) A Methylophilus bacterium (II), into which (I) or (II) is introduced in an expressible form and has an ability to produce L-lysine or L-arginine; and (3) Producing L-lysine or L-arginine comprising culturing (II) in a medium and collecting L-lysine or L-arginine from the culture.

BIOTECHNOLOGY - Preferred DNA: (I) encodes a mutant protein which substantially consists only of the hydrophobic helixes. Preferably, the mutant protein has all of the six hydrophobic helixes. (I) encodes a peptide containing the first to third hydrophobic helixes from the N-terminus and a peptide containing the fourth to sixth hydrophobic helixes from the N-terminus. Most preferably, (I) encodes LysE protein from a coryneform bacterium. The methanol assimilating bacterium as described above is a Methylophilus bacterium.

USE - (I) is used for encoding a protein which facilitates excretion of L-lysine, L-arginine or both of these L-amino acids to outside of a cell of a **methanol** assimilating bacterium when (I) is introduced into the bacterium. (II) is useful for producing L-lysine or L-arginine (claimed).

ADVANTAGE - The promotion of excretion of the L-amino acids from the inside of the cell to the outside to the cell is observed as increased concentrations of the L-amino acids accumulated in the medium during the culture of the methanol assimilating bacterium containing (I) compared with the concentrations provided by the methanol assimilating bacterium not containing (I). Further, the promotion of excretion of the L-amino acids to outside of a cell may also be observed as decrease of intracellular concentrations of the L-amino acids when (I) is introduced into a methanol assimilating bacterium.

EXAMPLE - An lysE gene which was a homologous gene of the gene facilitating excretion of L-lysine known for corynebacterium bacteria, was cloned from Brevibacterium bacterium. Construction of pRSlysE was carried out as follows. In order to introduce lysE into a Methylophilus bacterium a known plasmid pRS International Patent Publication in Japanese (Kohyo) No.3-501682 was used to construct a plasmid pRSlysE for expression of lysE. pRS is a plasmid having the vector segment of the pVIC40 plasmid International Patent Publication WO90/04636, International Patent Publication in Japanese No.3-501682 and obtained from pVIC40 by deleting a DNA region encoding the threonine operon contained in the plasmid. The plasmid pVIC40 was derived from a broad host spectrum vector plasmid pAYC32 Chistorerdov, A.Y., Tsygankov, Y.D., Plasmid, 1986, 16, 161-167 which is a derivative of RSF1010. First, a plasmid pRStac having the tac promoter was constructed from pRS. The pRStac plasmid was constructed as follows. The pRS vector was digested with restriction enzymes EcoRI and PstI and added with a phenol/chloroform solution and

mixed with it to terminate with the reaction. After the reaction mixture was centrifuged, the upper layer was collected and DNA's were collected by ethanol precipitation and separated. A DNA fragment of 8 kbp was collected, the tac promoter region was amplified by polymerase chain reaction (PCR) using the pKK223-3 plasmid (expression vector) as a template. The DNA fragment containing the amplified tac promoter was purified and then digested at EcoRI and EcoT221 sites. Then, the reaction mixture was added with a phenol/chloroform solution and mixed after the reaction mixture was centrifuged the upper layer was collected and DNA's were collected by ethanol precipitation and separated. A DNA fragment of about 0.15 kbp was collected. The digestion product of pRS vector and the tac promoter region fragment prepared as described above were ligated. The ligation reaction solution was used to transform Escherichia coli JM109 competent cells. The cells were plated on LB agar medium containing 20 mg/l of streptomycin and incubated overnight at 37 degreesC. The colonies appeared on the agar medium were each inoculated into LB liquid medium containing 20 mg/l of streptomycin and cultured at 37 degreesC for 8 hours with shaking. Plasmid DNA was extracted from each culture and structure of each plasmid was confirmed by digestion with restriction enzymes to obtain pRStac. pRStac obtained as described above was digested with Sse83871 and SapI added with a phenol/chloroform solution and mixed with it to terminate the reaction. After the reaction mixture was centrifuged, the upper layer was collected and DNA's were collected by ethanol precipitation and separated on 0.8% agarose gel to obtain a DNA fragment of about 9.0 kbp. lysE gene fragment was also amplified by PCR using chromosome extracted from the Brevibacterium lactofermentum 2256 strain as a template. The obtained fragment was purified and then digested with Sse83871 and SapI. The reaction mixture was added with a phenol/chloroform solution and mixed with it to terminate the reaction. After the reaction mixture was centrifuged, the upper layer was collected and DNA's were collected by ethanol precipitation and further collected from 0.8% agarose gel. The digestion product of the pRStac vector and the lysE gene region fragment prepared as described above were ligated. This ligation reaction solution was used to transform E.coli JM109 competent cells. The cells were plated on LB agar medium containing 20 mg/l of streptomycin and incubated overnight at 37 degreesC. The colonies appeared on the agar medium were each inoculated into LB liquid medium containing 20 mg/l of streptomycin and cultured at 37 degreesC for 8 hours with shaking. Plasmid DNA was extracted from each culture broth by the alkali-sodium dodecyl sulfate (SDS) method and structure of each plasmid was confirmed by digestion with restriction enzymes and determination of nucleotide sequence to obtain pRSlysE. pRSdapA obtained as described above was introduced into Methylophilus methylotrophus strain. The obtained transformant was referred to as AS1/pRSdapA. Methylophilus methylotrophus AS1 strain introduced with pRSlysE24 was referred to as AS1/pRSlysE24 and M.methylotrophus AS1 strain introduced with the pRS plasmid was referred to as AS1/pRS. The intracellular L-amino acid concentration and the L

-amino acid concentration in culture supernatant of above strains were determined. With AS1/pRS1ysE24, L-lysine accumulation substantially equivalent to that of AS1/pRSdapA was observed in the medium. On the other hand, with AS1/pRS1ysE24, the intracellular L-lysine concentration was suppressed to a low level, and it was considered that L-lysine was excreted to outside of the cells due to the introduction of the lysE24 gene. Further, concentrations of other L-amino acids in the culture supernatant were also investigated. As a result, it was found that L-arginine accumulated in AS1/pRS1ysE24. Thus, it was found that lysE24 had excretion activity not only for L-lysine but also for L-arginine. (23 pages)

ACCESSION NUMBER: 2003-11377 BIOTECHDS

TITLE:

Novel DNA encoding variant of LysE protein from a coryneform bacterium, when introduced into **methanol** assimilating bacterium, facilitates excretion of L-lysine

and/or L-arginine to outside of a cell;

plasmid pRSlysE-mediated lysE gene transfer to Methylophilus sp. for amino acid **production** 

AUTHOR: GUNJI Y; YASUEDA H PATENT ASSIGNEE: AJINOMOTO CO INC

PATENT INFO: EP 1266966 18 Dec 2002 APPLICATION INFO: EP 2002-12539 5 Jun 2002

PRIORITY INFO: JP 2001-177075 12 Jun 2001; JP 2001-177075 12 Jun 2001

DOCUMENT TYPE: Patent LANGUAGE: English

OTHER SOURCE: WPI: 2003-241171 [24]

L4 ANSWER 11 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

TI Novel nitrilase polypeptide, useful for making (R) - or

(S)-ethyl-4-cyano-3-hydroxybutyric acid or (R)- or (S)-mandelic acid or (S)- or (R)-phenyl lactic acid derivative and for producing

pharmaceutical composition, and food additive;

vector-mediated recombinant protein gene transfer and expression in host cell for use in pharmaceutical and food industry and peptidomics 2003-10320 BIOTECHDS

AB DERWENT ABSTRACT:

AN

NOVELTY - An isolated polypeptide (I) comprising consecutive amino acids having a sequence at least 50% identical to any one of the 192 (300-400 residues long) amino acid sequences (S1) as given in the specification, and having nitrilase activity, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid (II) comprising consecutive nucleotides having a sequence at least 50% identical to any one of 192 (900-1100 base pairs long) sequences (S2) all given in the specification, or having a sequence at least 79% identical to a fully defined sequence (S3) of 1026 nucleotides as given in the specification, where the nucleic acid encodes a polypeptide having nitrilase activity; (2) a fragment of (II) encoding a polypeptide having nitrilase activity; (3) an isolated nucleic acid complementary to (II); (4) an isolated nucleic acid that hybridizes to (II) under stringent conditions; (5) a nucleic acid probe (III) comprising about 15-50 nucleotides, where at least 15 contiguous nucleotides are at least 50% complementary to a nucleic acid target region within (S2); (6) a nucleic acid vector (IV) capable of replication in a host cell, comprising (II) or (III); (7) a host cell (V) comprising (II) or (III); (8) a host organism (VI) comprising (V); (9) an isolated nucleic acid (VII) encoding a polypeptide comprising consecutive amino acids having a sequence at least 50% identical to (S1), where the polypeptide has nitrilase activity; (10) an isolated nucleic acid (VIII) encoding a polypeptide comprising at least 10 consecutive amino acids having a sequence identical to a portion of (S1); (11) a fragment of (I) which is at least 20 amino acids in length, and has nitrilase activity; (12) a peptidomimetic (IX) of (I) or its fragment having nitrilase activity; (13) a codon-optimized polypeptide of (I) or its fragment, having nitrilase activity, where the codon usage is optimized for a particular organism or cell; (14) a purified antibody (X) that specifically binds to (I) or its fragment having nitrilase activity; (15) a fragment of (X) which specifically binds to (I); (16) a liquid or dry enzyme preparation (XI) which comprises (I); (17) a composition comprising (I), (II), (III), (VII) or (IX), having nitrilase activity or their combination; (18) making (I); (19) a computer readable medium which stores (S1) or (S2); (20) a computer system comprising a processor and a data storage device which stores (S1) and/or (S2); (21) identifying a feature in a sequence which comprises (S1), (S2) or (S3), involves inputting the sequence into computer, running a sequence feature identification program on the computer, and identifying the feature in the sequence; (22) assay for identifying functional variant of polypeptide, involves obtaining the variant of (I), contacting the variant with the substrate having cyanohydrine or aminonitrile moiety,

measuring amount of reaction product produced by each of the variant, and identifying the variant which is capable of producing nitrilase reaction product; (23) an isolated polypeptide exhibiting nitrilase activity, and comprising three consensus subsequences, such as FPETF, RRKLXPT and LXCWEHXXP, respectively; and (24) an isolated polypeptide exhibiting nitrilase activity, comprising three consensus subsequences, such as FPEXX, XRKLXPT and LXCWEXXXP, respectively; (25) a method for making (R)or (S)-ethyl-4-cyano-3- hydroxybutyric acid; (26) a method for making an (R)-mandelic acid comprising (R)-2-chloromandelic acid; (27) a method for making (S)-mandelic acid comprising (S)-methyl benzyl cyanide; (28) a method for making (S) - or (R) -phenyl lactic acid derivative, by admixing phenyllactocyanonitrile with (I), its fragment or (IX); (29) a method for generating a nucleic acid variant encoding a polypeptide having nitrilase activity, having an altered biological activity from that which naturally polypeptide, which involves modifying (II) or (III) by substitution, deletion or addition mutations;

BIOTECHNOLOGY - Preparation: (I) is prepared by introducing a nucleic acid encoding the polypeptide in a host cell under conditions that permit production of the polypeptide by the host cell, and recovering the polypeptide so produced (claimed). Preferred Polypeptide: (I) comprises consecutive amino acids having preferably 98% identity to (S1). Most preferably, (I) comprises the sequence of (S1). (I) or its fragment of (IX) is affixed to a solid support such as a gel, resin, polymer, ceramic, glass, microelectrode or their combinations. Preferred Nucleic Acid: (II) comprises consecutive nucleotides having at least 96%-100% identical to (S2) or (S3), or is substantially identical to (S2), or has a sequence identical to (S2). In (III), the 50 consecutive nucleotides are preferably 98-100% complementary to the nucleic acid target region. The nucleic acid consists essentially of 20-50 nucleotides. (VII) encodes a polypeptide comprising consecutive amino acids having a sequence which preferably 95-100% identity to (S1), or comprises consecutive amino acids having the sequence of (S1). Most preferably, (VII) encodes a polypeptide comprising at least 10 consecutive amino acids having a sequence identical to a portion of (S1). (II), (III) or (VII) is affixed to a solid support. Preferred Organism: (VI) comprises a gram negative bacteria (e.g., Escherichia coli or Pseudomonas fluorescens), gram positive bacteria (e.g., Streptomyces diversa, Lactobacillus gasseri, Lactococcus lactis, Lactococcus cremoris or Bacillus subtilis) or an eukaryotic organism e.g., Saccharomyces cerevisiae, Schizosaccharomyces pombe, Pichia pastoris, Kluyveromyces lactis, Hansenula polymorpha or Aspergillus niger. Preferred Preparation: (XI) is affixed to a solid support. Preferred Method: The method of (29) further comprises assaying the polypeptides encoded by the modified nucleic acids for altered nitrilase activity. The modifications are made by polymerase chain reaction (PCR), error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, etc.

USE - (I) or its fragment or (IX) is useful for hydrolyzing a nitrile to a carboxylic acid under aqueous conditions of pH 8 and/or at 37-45 degrees Centigrade; hydrolyzing a cyanohydrin moiety or an aminonitrile moiety of a molecule; making a chiral alpha-hydroxy acid molecule or a chiral amino acid molecule; and making a composition or its intermediate, which involves admixing a precursor (comprising a cyanohydrin moiety or an aminonitrile moiety) of the composition or its intermediate, with (I), or its fragment or (IX), such that the cyanohydrin or aminonitrile moiety is hydrolyzed thereby making the composition or its intermediate. The composition or its intermediate preferably comprises (S)-2-amino-4-phenyl butanoic acid or an L -amino acid. The method is also useful for making a composition comprising a food additive or a pharmaceutical drug. (I) is useful for making (R) - or (S) -ethyl-4-cyano-3- hydroxybutyric acid. (I) is also useful for making an (R)-mandelic acid comprising (R)-2-chloromandelic acid, for making (S)-mandelic acid comprising

(S)-methyl benzyl cyanide, or for making (S)- or (R)-phenyl lactic acid derivative. (II) or (III) is useful for generating a nucleic acid variant encoding a polypeptide having nitrilase activity, or having an altered biological activity from that which naturally polypeptide. (II), (III) or (VII) is useful for making a polynucleotide from two or more nucleic acids, by identifying regions of identity and regions of diversity between the two or more nucleic acids, providing a set of oligonucleotides which correspond in sequence to at least two of the two or more nucleic acids, and extending the oligonucleotides with a polymerase, to make the polynucleotide. (I), (II), (III) or (VII) is useful for screening assay for identifying nitrilase activity. (I) or its fragment or (IX) is useful in an industrial process for producing a pharmaceutical composition, food additive, for catalyzing the breakdown of waste, producing a detergent, producing a food product, or producing a drug intermediate which involves use of the polypeptide to hydrolyze a hydroxyglutarylnitrile substrate. Preferably, the drug intermediate production process is the process for the production of LIPITOR. (I) or its fragment or (IX) is also useful for modifying a molecule and for identifying a modified compound. Fragment of (I) is useful for identifying a functional fragment or polypeptide, which is capable of producing a nitrilase reaction product. (II) or (III) is useful for preparing a transgenic organism (all claimed).

ADVANTAGE - Using (I), dynamic resolution conditions can be established due to racemization of substrate under aqueous conditions. (I) has increased activity and stability at increased pH and temperature.

EXAMPLE - For each library to be screened for nitrilases, an infection was set up as follows. 5 ml of an OD(600)nm = 1 resuspension of SEL700 cells and 1 ml of the phagemid library to be screened were combined. The combination was incubated in a 37 degrees Centigrade waterbath for 45 min. Using the infection, serial dilutions were made in 10 mM MgSO4, using 10 microlitres aliquots of the infection. 60 microlitres of the following dilutions (10 (to the power of -1) dilutions for 10 (to the power of 5) colony forming unit (cfu)/ml of library), (10 (to the power of -1), 10 (to the power of -2) dilutions for 10 (to the power of 6) cfu/ml of library), and (10 (to the power of -2) and 10 (to the power of -3) dilutions for 10 (to the power of 7) cfu/ml of library). The cells in the infection were centrifuged. The supernatant was decanted from the resulting pellets. The cells were resuspended in residual liquid. All of the resuspended cells were deposited onto a single large LB-Kan50 plate. All plates were incubated at 30 degrees Centigrade overnight. The cells of each infection plate were resuspended with 4 mls 10 mM MgSO4. The resuspensions were placed in a tube. The remaining cells on each plate were resuspended with 3 mls 10 mM MgSO4 and combined with the first resuspension from the same plate. The volume of each tube was brought to 12 ml with 10 mM MgSO4, the tubes were vortexed. The tubes were centrifuged. The supernatant was decanted from each resuspension. The washed cells in each tube were resuspended with 10 ml 20 mM MgSO4. For each resuspension, selection cultures were set up using the following processes. The nitrilase selection medium was prepared, using 1 x M9 medium with 0.2% glucose, no nitrogen and 50 micrograms/ml kanamycin, 5 ml of the medium was aliquoted into a 50 ml screw top conical tube, 25 microlitres of the stored resuspension was added to the tube, 5 microlitres adiponitrile was added to the tube, to bring the final concentration to 8.8 mM. Additional nitrile substrates may be used, in place of adiponitrile. Ten (10) microlitres of selection culture with growth was streaked out onto a small LB-kan50 plate and allowed to grow for 2 nights at 30 degrees Centigrade. Five isolated cfu were picked and each was grown in 2 ml nitrilase selection medium at 30 degrees Centigrade. Each culture was monitored and was removed. One (1) ml of culture was used to do a plasmid preparation and was eluted with 40 microlitres elution buffer. Five to eight (5-8) microlitres DNA was cut with PstI/XhoI or SacI/KpnI restriction enzymes to remove insert from vector. A restriction fragment length polymorphism (RFLP) determination

was carried out to identify the size of the insert. The insert was sequenced. Nitrilases of the invention were screened against target substrates. Of those showing hydrolytic activity in primary screen, enzymes with enantioselectivities above 20% enantiomeric excess (ee) were selected for further characterization. Those enzymes were selected base on, having activity against one of the substrates of interest and exhibition of greater than 35% ee (enantiomeric excess). The products used for screening were, D-Phenylglycine, L-Phenyllactic acid, (R) 2-chloromandelic acid, (S)-Cyclohexylmandelic acid, L-2methylphenylglycine, (S)-2-amino-6-hydroxy hexanoic acid, and 4-methyl-L-leucine. Of the enzymes identified active on phenylglycinonitrile, the enantioselectivity of several enzymes was shown to remain above the success criterion of 35% ee. The preliminary characterization data indicated that some of the enzymes exhibited high enantioselectivities for D-phenylglycine, with corresponding conversion to product of 40-60%. From screening of the nitrilases, against target substrate (R)-2-chloromandelic acid, it was found that higher temperatures and neutral pH appeared to lead to the highest activity for the active enzymes. For the majority of the nitrilases, the enantioselectivity also increased at higher temperatures, particularly 38 degrees Centigrade. The enzymes retained their activity in the presence of up to 25% methanol or 10% isopropanol, in many of these cases, the enantioselectivity was also enhanced. Activity in biphasic systems was largely comparable to aqueous conditions, particularly with hexane as the non-aqueous phase, varying tolerances to toluene were observed between the different nitrilases. (560 pages)

ACCESSION NUMBER: 2003-10320 BIOTECHDS

TITLE: Novel nitrilase polypeptide, useful for making (R) - or

(S) -ethyl-4-cyano-3-hydroxybutyric acid or (R) - or

(S)-mandelic acid or (S)- or (R)-phenyl lactic acid

derivative and for producing pharmaceutical composition, and

food additive;

vector-mediated recombinant protein gene transfer and expression in host cell for use in pharmaceutical and food

industry and peptidomics

AUTHOR: MADDEN M; DESANTIS G; CHAPLIN J A; WEINER D P; MILAN A; CHI

E; SHORT J M; BURK M

PATENT ASSIGNEE: DIVERSA CORP; MADDEN D
PATENT INFO: WO 2003000840 3 Jan 2003

APPLICATION INFO: WO 2002-US15983 15 May 2002

PRIORITY INFO: US 2002-351336 22 Jan 2002; US 2001-300189 21 Jun 2001

DOCUMENT TYPE: Patent LANGUAGE: English

OTHER SOURCE: WPI: 2003-201417 [19]

L4 ANSWER 12 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

TI Efficient production of L-amino acids e.g. L-lysine by

fermenting transformed Methylopillus bacterium with enhanced dihydrodipicolinate-synthase and aspartokinase activities or casamino acid requirement using methanol;

plasmid RSF and plasmid pRS-lysC80-mediated aspartate-kinase, aspartic semi-aldehyde-dehydrogenase, dihydropicolinate-reductase or diaminopimelate decarboxylase gene transfer

AN 2001-02341 BIOTECHDS

AB

A bacterium of Methylophilus sp., preferably Methylophilus methylotrophus, can produce an L-amino acid efficiently, which is a transformed bacterium with enhanced enzyme activity, is new. Also claimed are: a process for preparing an L-amino acid; a DNA having a 1,981 bp sequence encoding a 409 amino acid protein sequence having aspartate-kinase (EC-2.7.2.4) activity; a DNA having a 1,452 bp sequence encoding a 370 amino acid protein sequence with aspartic semi-aldehyde-dehydrogenase activity; a DNA having a 3,098 bp sequence encoding a 296 amino acid

protein sequence with dihydropicolinate-synthase (EC-4.2.1.52) activity; a DNA having a 3,390 bp sequence encoding a 286 amino acid protein sequence with dihydropicolinate-reductase activity; and a DNA having a 2,566 bp sequence encoding the 415 amino acid protein sequence with diaminopimelate-decarboxylase (EC-4.1.1.20) activity. The method is for the **production** of L-amino acids including L-lysine, L-valine, L-leucine, L-isoleucine or L-threonine. In an example, a Methylophilus sp. was transformed with plasmid RSF containing mutated dapA and expression plasmid pRS-lysC80. (92pp)

ACCESSION NUMBER: 2001-02341 BIOTECHDS

TITLE: Efficient production of L-amino acids e.g. L-lysine by fermenting transformed Methylopillus bacterium with

enhanced dihydrodipicolinate-synthase and aspartokinase

activities or casamino acid requirement using

methanol;

plasmid RSF and plasmid pRS-lysC80-mediated

aspartate-kinase, aspartic semi-aldehyde-dehydrogenase,

dihydropicolinate-reductase or diaminopimelate

decarboxylase gene transfer

AUTHOR: Gunji Y; Yasueda H; Sugimoto S; Tsujimoto N; Shimaoka M;

Miyata Y; Oba M

PATENT ASSIGNEE: Ajinomoto LOCATION: Tokyo, Japan.

PATENT INFO: WO 2000061723 19 Oct 2000 APPLICATION INFO: WO 2000-JP2295 7 Apr 2000

PRIORITY INFO: JP 1999-368097 24 Dec 1999; JP 1999-103143 9 Apr 1999

DOCUMENT TYPE: Patent LANGUAGE: Japanese

OTHER SOURCE: WPI: 2000-672679 [65]

L4 ANSWER 13 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

TI New (4S)-hydroxy-3-methyl-2-ketopentanoic acids;

stereospecific hydroxymethylketopentanoic acid production by

L-amino-acid-oxidase or Morganella

morganii for use as a flavor

AN 1995-01122 BIOTECHDS

4S-hydroxy-3-methyl-2-ketopentanoic acid of formula (I) is new. Also new is production of stereospecific 5S-3-hydroxy-4,5-dimethyl-2(5H)-furanone of formula (III), which involves lactonizing (I). (I) is produced by reacting L-4-hydroxyisoleucine (II) with a microorganism with L-amino-acid-oxidase (EC-1.4.3.2) activity or with the enzyme alone. (I) is useful as a flavor precursor, while (III) is a flavor. In an example, Morganella morganii DSM 30117 was grown in a fermentor for 20 hr at 30 deg and pH 7 in culture medium containing 10 g/l glucose, 10 g/l casein hydrolyzate and salts. The cells were centrifuged, washed with 0.02 M sodium phosphate buffer (pH 7) and the

cell solution was reacted with 7.7 mmol L-4-hydroxyisoleucine (prepared from 4 kg seeds of Trigonella foenum graecum L.) and the mixture was incubated at 30 deg for 6 hr with agitation. The cells were separated from the mixture by centrifugation and the residue was lyophilized. The residue was extracted with methanol and 2-keto-3-methyl-4-

hydroxyvaleric acid sodium salt was prepared by adding methyl-tert. butyl ether. (10pp)

ACCESSION NUMBER: 1995-01122 BIOTECHDS

TITLE: New (4S)-hydroxy-3-methyl-2-ketopentanoic acids;

stereospecific hydroxymethylketopentanoic acid

production by L-amino-

acid-oxidase or Morganella morganii for use as a

flavor

AUTHOR: Lerch K

PATENT ASSIGNEE: Givaudan-Roure

PATENT INFO: EP 623580 9 Nov 1994

APPLICATION INFO: EP 1994-106577 27 Apr 1994

PRIORITY INFO: CH 1993-1394 6 May 1993

DOCUMENT TYPE: Patent LANGUAGE: German

OTHER SOURCE: WPI: 1994-343246 [43]

L4 ANSWER 14 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

TI L-amino acid production e.g.

L-threonine, L-lysine, L-valine, L-alanine, L-aspartic acid, L-glutamic acid, L-isoleucine, L-leucine;

by beta-fluoropyruvic acid- 3-bromopyruvic acid-resistant

Methylobacillus sp. using a methanol C-source

AN 1994-11177 BIOTECHDS

AB L-amino acid is prepared by culturing

Methylobacillus sp. (which is resistant to halogenated pyruvic acid) and collecting the amino acid from the culture medium. Preferably: (1) the halogenated pyruvate is beta-fluoropyruvic acid or 3-bromopyruvic acid;

(2) the medium contains methanol as a principle C-source; and

(3) the L-amino acid is L-threonine,

L-lysine, L-valine, L-alanine, L-aspartic acid, L-glutamic acid,

L-isoleucine or L-leucine. This method allows efficient

production of an L-amino acid from a cheap methanol C-source. (6pp) ACCESSION NUMBER: 1994-11177 BIOTECHDS

TITLE: L-amino acid production

e.g. L-threonine, L-lysine, L-valine, L-alanine, L-aspartic

acid, L-glutamic acid, L-isoleucine, L-leucine;

by beta-fluoropyruvic acid- 3-bromopyruvic acid-resistant

Methylobacillus sp. using a methanol C-source

PATENT ASSIGNEE: Kyowa-Hakko

PATENT INFO: JP 06133788 17 May 1994 APPLICATION INFO: JP 1992-291140 29 Oct 1992 PRIORITY INFO: JP 1992-291140 29 Oct 1992

DOCUMENT TYPE: Patent LANGUAGE: Japanese

OTHER SOURCE: WPI: 1994-196169 [24]

L4 ANSWER 15 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

TI **Production** of stereospecific N-acyl amino acid; using Aspergillus and Penicillium aminoacylase

AN 1990-08405 BIOTECHDS

An acyl donor and L-aminoacylase (EC-3.5.1.14) are mixed to form a racemic solution, which acylates **L-amino acid** in the presence of an organic solvent at 35-40 deg and pH 6-8 to form N-acyl-**L-amino acid**. The method is simple and gives good yields of high purity products. The acyl donor is preferably an acetate or propionic acid salt. The organic solvent is preferably **methanol**, ethanol, dimethyl sulfoxide, acetone, dioxane, glycerine, ethylene glycol, isopropanol, acetonitrile or tetrahydrofuran. The concentration of the organic solvent is preferably

tetrahydrofuran. The concentration of the organic solvent is preferably 10-90%, especially 20-80%. The aminoacylase is preferably derived from

an Aspergillus or a Penicillium sp. (4pp)

ACCESSION NUMBER: 1990-08405 BIOTECHDS

TITLE: **Production** of stereospecific N-acyl amino acid;

using Aspergillus and Penicillium aminoacylase

PATENT ASSIGNEE: Amano-Pharm.

PATENT INFO: JP 02072897 13 Mar 1990 APPLICATION INFO: JP 1988-224088 7 Sep 1988 PRIORITY INFO: JP 1988-224088 7 Sep 1988

DOCUMENT TYPE: Patent LANGUAGE: Japanese

OTHER SOURCE: WPI: 1990-121058 [16]

TI Microbial and enzymatic processes for the **production** of biologically active amino acids;

L-amino acid and D-amino acid production (conference paper)

1989-09694 BIOTECHDS

ΑN

AB

Tyrosine-phenol-lyase (EC-4.1.99.2), tryptophanase (EC-4.1.99.1), cystathionine-gamma-lyase (EC-4.4.1.1), O-acetyl-L-serine-sulfhydrase and beta-chloro-D-alanine-dehydrochlorinase catalyzed the synthesis of L-tyrosine, L-tryptophan, L-cysteine, D-cysteine and related amino acids in significantly high yields. Cystathionine-gamma-lyase and O-succinylhomoserine-thiol-lyase (EC-4.2.99.9) catalyzed the synthesis of cystathionine and related amino acids in yields of nearly 100% under suitable reaction conditions. L-serine was synthesized from glycine and methanol by methanol-dehydrogenase and serine-hydroxymethyltransferase. Hyphomicrobium methylovorum strain KM 146 was selected as a serine producer. Under optimum reaction conditions, L-serine was produced in a 20% yield from 100 mg/ml glycine. A glycine-resistant mutant showed 1.5-fold productivity higher than the parent. A new process was developed for the production of D-p-hydroxyphenylglycine, an important component of semisynthetic penicillins and cephalosporins. Dihydropyrimidinase was used and the

ACCESSION NUMBER: 1989-09694 BIOTECHDS

TITLE: Microbial and enzymatic processes for the production

process was the most economical for industrial production.

of biologically active amino acids;

L-amino acid and D-amino

acid production (conference paper)

AUTHOR: Yamada H

ref)

LOCATION: Department of Agricultural Chemistry, Kyoto University, Kyoto

606, Japan.

SOURCE: Eur.Congr.Biotechnol.; (1987) Vol.4, 689-92

DOCUMENT TYPE: Journal LANGUAGE: English

L4 ANSWER 17 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

TI Microbial and enzymatic process for the **production** of optically active amino acids;

amino acid preparation using enzyme (conference abstract)

AN 1989-06906 BIOTECHDS

AB

Stereospecific amino acid preparation using enzymes was studied. Tyrosine-phenol-lyase (EC-4.1.99.2), tryptophanase (EC-4.1.99.1), cystathionine-gamma-lyase (EC-4.4.1.1), O-acetylserine-thiol-lyase (EC-4.2.99.8) and 3-chloro-D-alanine-dehydrochlorinase (EC-4.5.1.2) were used to synthesize aromatic and sulfur-containing amino acids through beta-replacement and the reverse of alpha, beta-elimination reactions. Cystathionine-gamma-lyase and O-succinylhomoserine-thiol-lyase (EC-4.2.99.9) were used to synthesize cystathionine and related amino acids from L-acyl-L-homoserine and cysteine analogs, in almost 100% yields. L-serine was synthesized from glycine and methanol by phosphoribosylglycinamide-formyltransferase (EC-2.1.2.2) of methylotrophic bacteria. For synthesis of D-phenylglycine and related D-amino acids, dihydropyrimidinase (EC-3.5.2.2) catalyzed ring-opening of dihydropyrimidines, and hydrolyzed 5-monosubstituted hydantoins. Hydantoins were hydrolyzed to D-forms of the N-carbamoyl glycine derivatives. These N-carbamyl amino acids were transformed to D-amino acids by treatment with NaNO2 under acidic conditions. (0 ref)

ACCESSION NUMBER: 1989-06906 BIOTECHDS

TITLE: Microbial and enzymatic process for the **production** 

of optically active amino acids;

amino acid preparation using enzyme (conference abstract)

AUTHOR: Yamada H

LOCATION: Department of Agricultural Chemistry, Kyoto University, Kyoto

606, Japan.

SOURCE: Eur.Congr.Biotechnol.; (1987) Vol.2, 19

DOCUMENT TYPE: Journal LANGUAGE: English

L4 ANSWER 18 OF 18 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN Racemization of amino acid esters by aromatic aldehydes in basic non-aqueous solvents;

potential use with hydrolase for the stereospecific **production** of L-amino acids

AN 1985-03652 BIOTECHDS

The racemization of optically active leucine methyl ester in the presence AB of pyridoxal or pyridoxal 5-phosphate was studied in water at neutral pH and in methanol at different concentrations of triethylamine. In water, the racemization rate with pyridoxal 5-phosphate was 10 times faster than with pyridoxal. In methanol containing triethylamine, the effectiveness of pyridoxal and pyridoxal 5-phosphate were of the same order and both were more effective than in water. Racemization of amino acid esters by combined pyridoxal and triethylamine catalysis occurred in diverse organic media such as monohydric alcohols and halogenated hydrocarbons. Both the aromatic ring N and the hydroxyl group at position 2 (as in pyridoxal) were important for high catalytic properties in the aromatic aldehydes. Pyridoxal was immobilized by covalent linking to an insoluble polyaryldiazonium reagent and suspended in basic methanol solutions of amino acid ester. Racemization took place in the heterogeneous system. The solid catalyst for racemization will be used in combination with a chiral-specific hydrolase, also insolubilized, for the stereospecific production of L-amino acids. (5 ref)

ACCESSION NUMBER: 1985-03652 BIOTECHDS

TITLE: Racemization of amino acid esters by aromatic aldehydes in

basic non-aqueous solvents;

potential use with hydrolase for the stereospecific

production of L-amino acids

AUTHOR: Pugniere M; San Juan S; \*Previero A

LOCATION: INSERM, 60 rue de Navacelles, 34100 Montpellier, France.

SOURCE: Biotechnol.Lett.; (1985) 7, 1, 31-36

CODEN: BILED3

DOCUMENT TYPE: Journal LANGUAGE: English

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FILE 'MEDLINE, SCISEARCH, EMBASE, WPIDS, FSTA, JICST-EPLUS, BIOSIS, BIOTECHDS' ENTERED AT 13:57:54 ON 09 JAN 2006

L2 1213 S L-AMINO ACID AND PRODUCTION
L3 3 S L2 AND L1
L4 18 S L2 AND METHANOL
E GUNJI, Y/AU

=> s 14 and (6-phosphogluconate dehydratase)
L5 0 L4 AND (6-PHOSPHOGLUCONATE DEHYDRATASE)

# Refine Search

## Search Results -

Terms	Documents
20040146974	1

US Pre-Grant Publication Full-Text Database US Patents Full-Text Database US OCR Full-Text Database **EPO Abstracts Database** Database: JPO Abstracts Database Derwent World Patents Index IBM Technical Disclosure Bulletins L8

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## **Search History**

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DB=U	SPT; PLUR=YES; OP=OR			
<u>L7</u>	L6 and l5	2	<u>L7</u>	
<u>L6</u>	gunji.in.	229	<u>L6</u>	
<u>L5</u>	L4 and methanol	114029	<u>L5</u>	
<u>L4</u>	L3 and 11	288018	<u>L4</u>	
<u>L3</u>	L-amino acid production	1165210	<u>L3</u>	
<u>L2</u>	(6-phosphogluconate dehydratase activity)	403367	<u>L2</u>	
<u>L1</u>	(6-phosphogluconate dehydratase activity)	403367	<u>L1</u>	

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<u>L6</u>	gunji.in.	229	<u>L6</u>		
<u>L5</u>	L4 and methanol	114029	<u>L5</u>		
<u>L4</u>	L3 and 11	288018	<u>L4</u>		
<u>L3</u>	L-amino acid production	1165210	<u>L3</u>		
<u>L2</u>	(6-phosphogluconate dehydratase activity)	403367	<u>L2</u>		
<u>L1</u>	(6-phosphogluconate dehydratase activity)	403367	<u>L1</u>		

**END OF SEARCH HISTORY** 

## **Hit List**

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Search Results - Record(s) 1 through 2 of 2 returned.

1. Document ID: US 6177541 B1

L7: Entry 1 of 2 File: USPT Jan 23, 2001

US-PAT-NO: 6177541

DOCUMENT-IDENTIFIER: US 6177541 B1

TITLE: Process for producing an isocyanurate derivative

DATE-ISSUED: January 23, 2001

INVENTOR-INFORMATION:

NAME CITY STATE ZIP CODE COUNTRY

Ikeda; HisaoFunabashiJPKoda; ToshinariFunabashiJPGunji; YasuhiroFunabashiJP

US-CL-CURRENT: <u>528/487</u>; <u>528/287</u>, <u>528/422</u>, <u>528/423</u>, <u>528/503</u>, <u>528/72</u>, <u>528/80</u>

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KOMC	Draw, Desc	emi

2. Document ID: US 5871843 A

L7: Entry 2 of 2 File: USPT Feb 16, 1999

US-PAT-NO: 5871843

DOCUMENT-IDENTIFIER: US 5871843 A

TITLE: Laminate and process for its production

DATE-ISSUED: February 16, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Yoneda; Takashige	Yokohama			JP
Fukawa; Makoto	Yokohama			JP
Morimoto; Takeshi	Yokohama			JP
Sato; Kazuo	Yokohama			JР
<u>Gunji</u> ; Fumiaki	Yokohama			JР
Nishimura; Hiromichi	Yokohama			JP
Takeda; Satoshi	Yokohama			JP
Hayashi; Yasuo	Yokohama			JP
Fujita; Hiroyuki	Kanagawa			JP

US-CL-CURRENT: 428/337; 427/165, 428/315.5, 428/428

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